

PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 36436PC01	FOR FURTHER ACTION		See Form PCT/IPEA/416
International application No. PCT/DK2005/000132	International filing date (<i>day/month/year</i>) 25.02.2005	Priority date (<i>day/month/year</i>) 26.02.2004	
International Patent Classification (IPC) or national classification and IPC C12N15/10, C12N13/00, C12N1/06, C12M1/33, C12M1/42, B01J19/00, B01L3/00			
Applicant THOMSEN BIOSCIENCE AS			
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 9 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p style="margin-left: 20px;">a. <input checked="" type="checkbox"/> <i>sent to the applicant and to the International Bureau</i>) a total of 3 sheets, as follows:</p> <p style="margin-left: 40px;"><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> (<i>sent to the International Bureau only</i>) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>			
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input checked="" type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input checked="" type="checkbox"/> Box No. VIII Certain observations on the international application</p>			
Date of submission of the demand 22.12.2005		Date of completion of this report 06.02.2006	
Name and mailing address of the international preliminary examining authority: <div style="display: flex; align-items: center;"> <div> European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016 </div> </div>		Authorized Officer Hornig, H Telephone No. +31 70 340-2620 <div style="text-align: right;"> </div>	

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PCT/DK2005/000132

Box No. 1 Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
 - ☐ international search (under Rules 12.3 and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4)
 - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements*** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

Description, Pages

1-31 as originally filed

Claims, Numbers

20-22 as originally filed

1-19 received on 22.12.2005 with letter of 22.12.2005

Drawings, Sheets

1/6-6/6 as originally filed

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
 - ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
 - ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

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Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
- ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
 - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-19
	No: Claims	
Inventive step (IS)	Yes: Claims	1-19
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-19
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

10/590630

IAP9 Rec'd PCT/PTO 23 AUG 2006

International application No.

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RE ITEM V

1 Reference is made to the following documents:

D1 : WO 99/57314 A (FRAUNHOFER-GESELLSCHAFT ZUR FOERDERUNG DER ANGEWANDTEN FORSCHUNG E.V;) 11 November 1999 (1999-11-11)

D2 : WO 2004/013329 A (IMP COLLEGE INNOVATIONS LTD [GB]; MANZ ANDREAS [GB]; FENNAH MELANIE [G] 12 February 2004 (2004-02-12)

D3 : WO 97/08293 A (SCIENTIFIC GENERICS LIMITED; MARTIN, SOPHIE, ELIZABETH, VICTORIA; BERG) 6 March 1997 (1997-03-06)

D4 : WO 99/28742 A (FRAUNHOFER GESELLSCHAFT ZUR FOERDERUNG DER ANGEWANDTEN FORSCHUNG E.V;) 10 June 1999 (1999-06-10)

D5 : US 5 891 694 A (ARISAWA ET AL) 6 April 1999 (1999-04-06)

The document WO0026405 was not cited in the international search report. A copy of the document is appended hereto.

D6: WO 00/26405 A (MESOSYSTEMS TECHNOLOGY, INC.[US]) 11 May 2000 (2000-05-11)

2 INDEPENDENT CLAIMS 1

2.1 The present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 seems to be new in the sense of Article 33(2) PCT. Document D1 discloses the isolation of Nucleic Acid with the use of Electric Fields by Means of a Sample Carrier in Chip Form (Example 5). According to D1 the field strengths ranges from 0.5 to 50, preferably from 5 to 30 kV/cm and pulse lengths respectively time constants of about

0.5[μs] to 50 ms as well as 1 to 1 000 000 pulses, preferably 1000 to 10 000 pulses, may be employed. For example the pulse form may be rectangular, decrease exponentially or sinus-shaped, preferably in the region of **radio frequency**. The electric field may be created in this case either by **alternating voltage** or by direct voltage.

2.2 The present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 seems to be new in the sense of Article 33(2) PCT. Document **D2** provides a DNA extraction method for extracting DNA from a sample including DNA-containing material (typically bacterial cells or cell debris), the method comprising the steps of providing a DNA extraction microchip including a main channel; delivering first and second parallel flows through the main channel, the first flow being of a sample including DNA-containing material and the second flow being of a separation medium ; applying an electroporation field to the first flow of sample including DNA-containing material such as to effect electroporation of DNA-containing material; and applying an electroseparation field across the main channel such as to effect electroseparation of DNA from the electroporated DNA-containing material in the first flow into the second, parallel flow.

2.3 The present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 seems to be new in the sense of Article 33(2) PCT. Document **D3** discloses a method for releasing intracellular material from cells which comprises: applying at most 50 V to a suspension containing the cell or cells. Also claimed is a method of producing single stranded nucleic acid which comprises releasing double stranded nucleic acid from cells as above using an electrode and denaturing the nucleic acid by applying a voltage to the suspension with the electrode to convert the double stranded nucleic acid to single stranded nucleic acid. Preferably, the voltage is from 0.5 to 50 volts with a strong preference for voltages in the lower part of this range e.g. from 0.5 to 15 volts, most preferably from 1 to 10 volts, wherein the voltage may be a DC voltage or an **AC voltage**. The voltage can be applied as a repeating pulse having a duration of up to 1 minute but preferably at 1 to 100 Hz. The electrodes by which the voltage is applied may preferably be spaced by 10 mm or less, e.g. 5 to 7 mm. However, **D3** teaches to optimise the conditions for producing denaturation of double-stranded DNA released from the cells, in which case a smaller electrode spacing will be desirable. To accomplish denaturation of released DNA, preferably the voltage is applied to the suspension between closely spaced

electrodes, preferably not spaced by more than 5 mm at their closest approach, e.g. by no more than 1.5 mm and most preferably by no more than 0.5 mm.

2.4 The present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 seems to be new in the sense of Article 33(2) PCT. Document **D4** discloses a method in which biological cells are broken down to extract the cell contents for analysis by being subjected to an electric field. The cells are brought into contact with a substance to encourage cell breakdown, before or while the electrical field affects the cells. The process is automated, with a simple apparatus and procedure. The biological cells, which are suspended in a liquid medium, are exposed to an electrical field strength between 5 and 100 kV/cm which is operated in pulsed mode with a pulse length of roughly 0,5 to 50 micro seconds. Furthermore, the voltage is applied to the suspension between closely spaced electrodes, preferably not spaced by more than 5 mm at their closest approach, e.g. by no more than 2 mm.

2.5 The present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 seems to be new in the sense of Article 33(2) PCT. Document **D5** discloses a method for recovery of nucleic acid which comprises: applying electric current to solution containing a microbe and nucleic acid and preferably protein are recovered from the solution. Escherichia coli was cultured and diluted by physiological saline water. A metal-coated hollow yarn membrane was immersed in the solution and 200 mA pulse current was applied to the membrane. **D5** teaches to send electricity using pulse waves and direct current. Impulse waves herein used means pulse waves which are differentiated. The solution in the membrane was sucked by a suction pump and the solution sucked was continuously fed to test tubes.

3 INDEPENDENT CLAIM 15

3.1 Document **D1** discloses a device 200 (Fig. 5), comprising a sample chamber 210 made up of four electrodes bundled in two opposite electrode pairs 220, 220' and a frame part 230 made of non-conducting materials. The sample carrier is rectangular in cross-section

and built to chip form factor, ie. is of a size of about 1 to 2 cm². Not shown are the lid and bottom part of the sample chamber 210. Frame part 230 and the four planar- shaped electrodes 220, 220' form the walls and delimit an interior volume that serves to take up the sample. The two opposite electrode pairs 220, 220' that each form one wall may be disposed at a distance of 100 nm to 5 mm and to possess an electrode diameter of about 1 cm. The sample chamber 210 possesses a frame part 230 having input units 260 and withdrawal units 270 that are equipped with filters. The input and withdrawal units 260, 270 are so fitted into connector-type non-conducting elements 280 of the frame part 230 that the sample carrier 200 can be filled and emptied.

3.2 The present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 seems to be new in the sense of Article 33(2) PCT. Document **D2** discloses a microchip for extracting DNA from a sample, comprises a main channel through which first and second parallel flows are delivered, and an electrode unit for applying electroporation or electro-separation fields to the sample. The system further comprises: a pulse generator for applying electroporation voltage pulses across the electroporation electrodes. The pulse generator is configured to generate electroporation voltage pulses at a voltage corresponding to an electroporation field strength of from about 500 V/cm to about 12.5 kV/cm, whereas the electroporation voltage pulses have a duration of from about 20 [mu]s to about 1 ms. Preferably, the electroporation voltage pulses have an interval of from about 2 ms to about 100 ms.

4 INDEPENDENT CLAIM 18

4.1 The present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claim 18 seems to be new in the sense of Article 33(2) PCT. Document **D2** discloses a microchip for extracting DNA from a sample, comprises a main channel through which first and second parallel flows are delivered, and an electrode unit for applying electroporation or electro-separation fields to the sample. The system further comprises: a pulse generator for applying electroporation voltage pulses across the electroporation electrodes. **D2** teaches, that the microfabricated DNA extraction device could be combined with other microfabricated DNA analysis devices, such as PCR, separation and sequencing devices, to provide a micromachined total analysis system

(u-TAS) which would allow for the rapid analysis of very small amounts of complex samples, which samples would require no pre-treatment, as DNA extraction would be achieved by the DNA extraction device as describes above.

5 INVENTIVE STEP OF CLAIMS 1, 15 AND 18

5.1 Document D6 describes a method for lysing a cell, to expose nuclear material. Said method comprises: creating an ionizing discharge; providing a surface on which the cell will be disposed either before or after the cell is lysed; and subjecting the cell to the ionizing discharge which causes the surface membrane of the cell to be ruptured so that nuclear material is exposed. The method enables the lysing of bacterial cells in a moist or dry (as opposed to wet) environment. The method also improves the yield of cell-nuclear material that is made available for identification and analyses.

Document D6, regarded as the closest state of the art differs from the subject-matter of the present application, that it lacks the technical feature that the bacterial spores are lysed in a liquid during exposure to the alternating electric field.

Therefore, in the light of D6 the subject-matter of claims 1, 15 and 18 are new under Art. 33(2) PCT.

In the light of the prior art, the problem to be solved by the present application may therefore be regarded as the provision of an alternative method and alternative device to extract biological material from a bacterial spore.

The solution as provided by the applicant comprises: (I) providing a sample chamber and a first and a second electrode, the first and the second electrode and the sample chamber being so positioned that at least a part of the sample chamber is between the first and the second electrode, b) providing a liquid sample in the sample chamber, which liquid sample comprises a bacterial spore, c) exposing said liquid sample to an alternating electric field in said sample chamber, said alternating electric field being provided by the first and the second electrode and having a sufficient amplitude so as to extract biological material from the bacterial spore, and d) optionally, performing an analysis on a part of the exposed

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liquid sample, said part comprising extracted biological material from the bacterial spore;
(ii) a chip for extracting biological material from a bacterial spore comprising a liquid sample comprising a bacterial spore, and (iii) a device for extracting biological material from a bacterial spore comprising a liquid sample comprising a bacterial spore.

In the light of **D6** in combination with any one of the prior art documents **D1-D5**, the subject-matter of claims 1, 15 and 18 comprises an inventive step under Art. 33 (3) PCT.

6 DEPENDENT CLAIMS 2-14,16-17 AND 19

Thus, claims 2-14,16-17 and 19 which are dependent on claims 1, 15 and 18 do also meet the requirements of the PCT in respect of novelty and/or inventive step (Article 33(2) and (3) PCT).

RE ITEM VIII

The dependent claim 10 does not meet the requirements of Art. 6 PCT in that the matter for which protection is sought is not clearly defined. The following statement:
"**..substantial form..**" does not enable the skilled person to determine which technical features are necessary to perform the stated function.

36436PC01

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PCT publication no.: WO 2005/083 078

Title: METHOD, CHIP, DEVICE AND SYSTEM FOR EXTRACTION OF BIOLOGICAL MATERIALS

Applicant: Thomsen Bioscience A/S

5 P&V reference: 36436PC01

Response to first Written Opinion dated 14 June 2005

10 AMENDED CLAIMS

1. A method for extracting biological material from a bacterial spore, the method comprising the steps of:

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a) providing a sample chamber and a first and a second electrode, the first and the second electrode and the sample chamber being so positioned that at least a part of the sample chamber is between the first and the second electrode,

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b) providing a liquid sample in the sample chamber, which liquid sample comprises a bacterial spore,

25

c) exposing said liquid sample to an alternating electric field in said sample chamber, said alternating electric field being provided by the first and the second electrode and having a sufficient amplitude so as to extract biological material from the bacterial spore, and

30

d) performing an analysis on a part of the exposed liquid sample, said part comprising extracted biological material from the bacterial spore.

2. The method according to claim 1, wherein the bacterial spore is selected from the genus *Bacillus* and/or the genus *Clostridium*.

35 3. The method according to any of the preceding claims wherein the bacterial spore is from the *Bacillus* group.

4. The method according to claim 3, wherein the bacterial spore is *Bacillus anthracis*.

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5. The method according to any of the preceding claims, wherein the first and a second electrode are separated by a distance being at the most 20 mm.

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6. The method according to any of the preceding claims, wherein the bacterial spore is either attached to and/or located between the first and the second electrode.
7. The method according to any of the preceding claims, wherein the frequency of the
5 alternating electric field is at the least 5 kHz.
8. The method according to claim 7, wherein the frequency of the alternating electric field is at the least 100 kHz.
- 10 9. The method according to any of the preceding claims, wherein the alternating electric field created by modulating the polarity of the first and the second electrode.
10. The method according to any of the preceding claims, wherein the alternating electric field has a substantial form chosen from the group consisting of: rectangular, sinusoidal,
15 saw-tooth, asymmetrical triangular, symmetric triangular; or any combination thereof.
11. The method according to any of the preceding claims, wherein the alternating electric field, in the frequency domain, comprises a least a first and a second frequency component.
- 20 12. The method according to any of the preceding claims, wherein the biological material comprises a component selected from the group consisting of a cell organelle, a genetic material, and a protein.
- 25 13. The method according to claim 12, wherein the genetic material comprises chromosomal DNA and/or plasmid DNA and/or any type of RNA.
14. The method according to claim 12, wherein the protein is selected from the group consisting of enzymes, structural proteins, transport proteins, ion channels, toxins,
30 hormones, and receptors.
15. A chip for extracting biological material from a bacterial spore, the chip comprising a sample chamber comprising:
- a sample chamber comprising a first opening in fluid connection with the surrounding
35 air and a second opening to form a fluid connection with a device,
 - a first and a second electrode positioned at opposing sides of the sample chamber, and
 - the sample chamber furthermore comprising a liquid sample comprise a bacterial spore.
- 40 16. The chip according to claim 15, wherein the first and a second electrode are positioned between the first and the second opening.
17. The chip according to claim 15 or 16, wherein bacterial spores located between the first and the second electrode.

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18. A device for extracting biological material from a bacterial spore, the device comprising:

- 5 - a chip site where the chip is to be located in order to be functionally associated with the device,
- an electrical interface between the device and the chip for applying an alternating electric field between the electrodes of the sample chamber, and
- a programmable unit comprising a software that effects that the device performs one or more actions selected from the group consisting of:
 - 10 - providing a liquid sample in sample chamber, which liquid sample comprises a bacterial spore,
 - exposing said liquid sample to an alternating electric field in said sample chamber, said alternating electric field having a sufficient amplitude so as to
 - 15 extract biological material from a bacterial spore, and
 - performing an analysis on a part of the exposed liquid sample which part comprises extracted biological material from the bacterial spore.
- 20 19. A system for extracting biological material from a bacterial spore, the system comprising a chip according to any of claims 15-17 functionally associated with a device according to claim 18.